PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SCB/58858001	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/00034	International filing date *(day/month/year)* 07.01.2003	Priority date *(day/month/year)* 07.01.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant NORCHIP A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.08.2003	13.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Aguilera, M Telephone No. +31 70 340-3897

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/00034

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-91 as originally filed

Sequence listings part of the description, Pages

1-67 as originally filed

Claims, Numbers

1-12 received on 11.06.2004 with letter of 10.06.2004

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 13-16

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☒ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☒ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

   see separate sheet

V. Reasoned statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

D1: WO 01 73135 A (MOLODYSKY EUGEN ;BIOSEARCH INTERNAT PTY LTD (AU); CORD JANET I (US) 4 October 2001 (2001-10-04)

D2: WO 99 29890 A (DIGENE CORP ;LORINCZ ATTILA T (US)) 17 June 1999

D3: LANHAM S ET AL: 'HPV detection and measurement of HPV 6, telomerase and survivin transcripts in colposcopy clinic patients' JOURNAL OF CLINICAL PATHOLOGY, LONDON, GB, vol. 54, no. 4, April 2001 (2001-04), pages 304-308.

D4: EP-A-0 662 518 (AMOCO CORP) 12 July 1995

D5: EP-A-0 373 352 (BEHRINGWERKE AG) 20 June 1990

D6: DE 44 31 174 A (DEUTSCHES KREBSFORSCH) 7 March 1996

D7: WO 94 26934 A (BROWN JANICE T ;BAXTER DIAGNOSTICS INC (US)) 24 November 1994

D8: SMITS H L ET AL: 'Application of the NASBA nucleic acid amplification method for the detection of human papillomavirus type 16 E6-E7 transcripts' JOURNAL OF VIROLOGICAL METHODS, AMSTERDAM, NL, vol. 54, no. 1, 1995, pages 75-81.

D9: CORNELISSEN M T E ET AL: 'UNIFORMITY OF THE SPLICING PATTERN OF THE E6/E7 TRANSCRIPTS IN HUMAN PAPILLOMAVIRUS TYPE 16-TRANSFORMED HUMAN FIBROBLASTS, HUMAN CERVICAL PREMALIGNANT LESIONS AND CARCINOMAS' JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 71, no. PART 5, 1 May 1990, pages 1243-1246.

D10: MCNICOL PATRICIA ET AL: 'Expression of human papillomavirus type 16 E6-E7 open reading frame varies quantitatively in biopsy tissue from different grades of cervical intraepithelial neoplasia' JOURNAL OF CLINICAL MICROBIOLOGY, vol. 33, no. 5, 1995, pages 1169-1173.

D11: JEON SAEWHA ET AL: 'Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: Implications for cervical carcinogenesis' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 92, no. 5, 1995, pages 1654-1658.

D12: LEONE GIONATA ET AL: 'Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA' NUCLEIC ACIDS RESEARCH, vol. 26, no. 9, 1998, pages 2150-2155.

Document D12 was not cited in the International Search Report, but it is cited by the applicant in page 28 of the description. For convenience, a copy of the document is appended hereto.

2.2 NOVELTY (Art. 33(2) PCT)

The subject-matter of present claims 1-12 is new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 Document D8 is considered to represent the most relevant state of the art and discloses a method of screening human subjects to assess their risk of developing cervical carcinoma, said method comprising screening the subject for expression of HPV E6 mRNA transcripts by isothermal amplification (see Abstract, and pages 76-80). The document describes the use of NASBA amplification of E6 transcripts in human patients of cervical carcinoma, and the detection of the amplification product *a posteriori* by Northern blot analysis and by ELGA (enzyme-linked gel assay).

2.3.2 The subject-matter of claim 1 differs in that the amplification product is detected in real time, i.e. simultaneously during amplification. This homogeneous assay avoids manipulation steps after amplification, rendering the process easier, faster and less prone to contamination.

2.3.3 The problem to be solved by the subject matter of claim 1 may therefore be regarded as providing an improved method of screening human subjects to assess their risk of developing cervical carcinoma. The solution would be to carry out a real-time detection of the amplification product. This is achieved by adding target-specific *molecular beacons* to the NASBA amplification reaction, and monitoring the fluorescence of the mixture during amplification.

2.3.4 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

2.3.4.1 The reagent kits and instrumentation for performing real-time NASBA detection were available commercially at the date of priority (see description, page 28, line 13), and, in view of the present disclosure, the optimization of said reagents for the particular use of E6 mRNA detection does not appear to involve an inventive step.

2.3.4.2 Moreover, D12 discloses the criteria for optimization of the composition of the probes, *molecular beacons,* and the NASBA reaction conditions, in order to achieve simultaneous amplification and detection of viral RNA (see page 2154, col. 2, par. 2, to page 2155, col. 1, par. 1). Applying these principles to another viral RNA, HPV E6 mRNA, lies within the scope of routine experimental design and optimization.

2.3.4.3 Furthermore, the authors of D12 refer in the Introduction to the methods of D8 (NASBA + Northern-blot or ELGA), and describe their method as an improvement over said techniques. The advantages of the new method which make it suitable for the present use, namely the rapidity, simplicity, minimization of contaminations, etc. are pointed out throughout the document. The combination of both documents would then be obvious to the skilled person faced with the present problem.

2.3.4.4 On the other hand, D8 reports that 2 of the 13 samples shown HPV-16 positive by PCR amplification of viral DNA were not detected by NASBA. However, this fact does not appear sufficient to deter the skilled person from improving the NASBA technique with routine modifications available, because the PCR is used to detect E6 viral DNA, and not E6 mRNA. In consequence, it does not provide comparative information with regard to the detection of transcriptionally active HPV, which is the purpose of the present methods. In addition, D8 strongly supports the usefulness of NASBA in the detection of E6 mRNA, and proposes it for the development of a diagnostic tool (see D8, page 80).

2.3.5 Since premalignant and malignant cells are considered as having "abnormal cell changes", the method of claim 2 is not inventive for the reasons explained

above.

2.3.6 Dependent claims 5-9 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, involve an inventive step, because they disclose merely some of the many straightforward possibilities from which the skilled person would select in order to solve the problem posed without the involvement of an inventive step. These features, pre-diagnoses patients, cancer stages, cancer-associated HPV types, etc. were well known at the date of priority, and they can be found, for example, in D4 (see page 11, lines 20-25), D9 (page 1245, column 1), D2 (page 10, line 23) or D5 (see page 2, line 16).

2.3.7 The kits of claims 10-12 are designed to carry out the methods of claims 1-9, which are not considered inventive (see above). Because the claimed products do not appear to provide any other technical effect that could involve an inventive step, the subject-matter of claims 10-12 is not considered inventive either. The specific sequences of primers and probes of the kit of claim 12 fall within the scope of routine experimental design that can be expected from the skilled person in view of the documents cited above.

2.3.8 Therefore, the subject-matter of claims 1-12 does not involve an inventive step in the sense of Article 33(3) and Rule 65(1)(2) PCT.

CLAIMS

1. An *in vitro* method of screening human subjects to assess their risk of developing cervical carcinoma, which method comprises screening the subject for expression of mRNA transcripts of the E6 gene of HPV and sorting the subject into one of two categories of risk for development of cervical carcinoma based on expression of E6 mRNA, wherein individuals positive for expression of E6 mRNA are scored as carrying integrated HPV or a modified episomal HPV genome and are therefore classified as high risk for development of cervical carcinoma, whereas individuals negative for expression of E6 mRNA are scored as not carrying integrated HPV or a modified episomal HPV genome and are therefore classified as no detectable risk for development of cervical carcinoma, characterised in that screening for E6 mRNA expression is carried using isothermal amplification in combination with real-time detection of the amplification product.

2. An *in vitro* method of identifying human subjects having abnormal cell changes in the cervix, which method comprises screening the subject for expression of mRNA transcripts of the E6 gene of HPV, wherein individuals positive for expression of E6 mRNA are identified as having abnormal cell changes in the cervix, characterised in that screening for E6 mRNA expression is carried using isothermal amplification in combination with real-time detection of the amplification product.

3. A method according to claim 1 or claim 2 wherein the isothermal amplification is NASBA, transcription-

mediated amplification, signal-mediated amplification of RNA or isothermal solution phase amplification.

4. A method according to claim 3 wherein screening for E6 mRNA expression is carried out using real-time NASBA.

5. A method according to any one of claims 1 to 4 wherein the human subjects are subjects previously identified as infected with human papillomavirus DNA in cells of the cervix.

6. A method according to any one of claims 1 to 5 wherein the human subjects are subjects having a previous diagnosis ASCUS, CIN 1 lesions or condyloma.

7. A method according to any one of claims 1 to 6 which comprises screening for E6 mRNA expression using a technique which is able to detect E6 mRNA from at least one cancer-associated HPV type.

8. A method according to claim 7 which comprises screening for E6 mRNA expression using a technique which is able to detect E6 mRNA from HPV types 16, 18, 31, 33, and preferably 45.

9. A method according to any one of claims 1 to 8 wherein individuals positive for expression of E6 mRNA from at least one of HPV types 16, 18, 31, 33 or 45 are scored as carrying integrated HPV.

10. A kit for use in the detection of mRNA transcripts of the E6 gene(s) of HPV, the kit comprising one or more

primer-pairs which enable amplification of a region of E6 transcripts from HPV types 16, 18, 31 and 33 by NASBA and one or more molecular beacon probes.

11. A kit according to claim 10 which comprises separate primer-pairs specific for each of HPV types 16, 18, 31 and 33.

12. A kit according to claim 10 or claim 11 which comprises one or more of, two or more of and preferably all of the following primer pairs and accompanying identification probes:
5' gatgcaaggtcgcatatgagCCACAGGAGCGACCCAGAAA and 5' AATTCTAATACGACTCACTATAGGGAGAAGGATTCCCATCTCTATATACTA with probe TATGACTTTGCTTTTCGGGA

5' gatgcaaggtcgcatatgagGAAAACGATGAAATAGATGGAG and 5' AATTCTAATACGACTCACTATAGGGAGAAGGGGTCGTCTGCTGAGCTTTCT with probe GAACCACAACGTCACACAATG

5' gatgcaaggtcgcatatgagACTGACCTCCACTGTTATGA and 5' AATTCTAATACGACTCACTATAGGGAGAAGGTATCTACTTGTGTGCTCTGT with probe GGACAAGCAGAACCGGACACATCCAA

5' GATGCAAGGTCGCATATGAGTATCCTGAACCAACTGACCTAT and 5' AATTCTAATACGACTCACTATAGGGAGAAGGTTGACACATAAACGAACTG with probe GGACAAGCACAACCAGCCACAGC.

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